Recovery of Salmonellae Following pH Adjusted Pre-Enrichment of Broiler Carcasses Treated with Trisodium Phosphate

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ABSTRACT Trisodium phosphate (TSP) has been reported to decrease the recovery of salmonellae from processed poultry carcasses. It has been suggested that the high pH and detergent-like properties of TSP solutions are responsible for the reduction in salmonellae recovery. This project was conducted to determine if controlling pH during salmonellae pre-enrichment alters the effect of TSP on salmonellae recovery. Carcasses were obtained from a commercial processing plant immediately after the final inside-outside carcass washer, prior to any other antimicrobial treatments, and before chilling. Carcasses were assigned to 1 of 4 treatment groups: 1) TSP and alkaline pre-enrichment, 2) TSP and neutral pre-enrichment, 3) non-TSP and alkaline pre-enrichment, 4) non-TSP and neutral pre-enrichment. Carcasses were placed into plastic bags with 500 mL of buffered peptone water (with or without pH adjustment) and shaken for 1 min. Preincubation pH of the rinsate was measured. Carcasses were incubated in the rinse at 37°C for 24 h, and incidence of salmonellae was determined. The pH of the preincubation rinsate was 8.4 for the TSP alkaline pre-enrichment, 7.2 for the TSP neutral pre-enrichment, 8.6 for the non-TSP alkaline pre-enrichment, and 7.1 for the non-TSP neutral pre-enrichment. Salmonellae were detected from 40% of the TSP alkaline pre-enrichment carcasses, 44% of the TSP neutral pre-enrichment carcasses, 54% of the non-TSP alkaline pre-enrichment carcasses, and 38% of the non-TSP neutral pre-enrichment carcasses. Neither TSP treatment nor pre-enrichment pH adjustment significantly influenced carcass salmonellae detection.

(*Key words*: pH, salmonella, trisodium phosphate, whole carcass enrichment)

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INTRODUCTION

Salmonellae are a common cause of foodborne gastroenteritis. The Center for Disease Control and Prevention (CDC) estimates that 1.4 million cases of salmonellosis, including over 500 fatalities, occur annually in the United States (CDC, 2004). Therefore, the reduction of salmonellae levels on food is an essential area in food safety research. Antimicrobial treatments such as chlorine and trisodium phosphate (TSP) are used to assist in the reduction of foodborne pathogens on processed poultry. TSP has been approved by the USDA for use in poultry processing plants (Bender and Brotsky, 1992; Giese, 1993).

The use of TSP as an antimicrobial wash can significantly decrease carcass salmonellae contamination levels (Bender and Brotsky, 1991; Kim et al., 1994a,b; Li et al., 1994; Lillard, 1994; Somers et al., 1994). However, the mechanisms of salmonellae reduction by TSP are not fully understood. The high pH of 12 (Teo et al. 1996; Sampathkumar et al., 2003), detachment of bacteria from the carcass surface (Lee, et al., 1994), and lipid removal from skin surfaces (Bender and Brotsky, 1992; Giese, 1992) have all been suggested as possible factors that lead to lower recovery of salmonellae from broiler carcasses.

In a commercial processing plant, carcasses are treated with 10% TSP (pH 12) at 24°C for 2 to 3 s, and the TSP is allowed to drip off the carcass for about 1 min before immersion chilling. Residual TSP is carried into the chiller and raises the chill water pH. The effectiveness of chlorine is decreased at elevated pH. Therefore, chiller water pH is often neutralized with carbon dioxide gas to improve the antimicrobial efficiency of chlorine. However, in most studies reporting salmonellae reduction by TSP, carcasses are treated for a longer period (10 to 30 min) or chill water pH is not neutralized before sampling for salmonellae (Bender and Brotsky, 1991; Li et al., 1994; Lillard, 1994; Somers et al., 1994). The objective of the current study was to compare the effect of TSP treatment with and without pH neutralization after treatment on the detection of salmonellae using whole carcass enrichment.

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Abbreviation Key: BPW = buffered peptone water; TSP = trisodium phosphate.

TABLE 1. Description of pre-enrichment treatments following either treatment with or without trisodium triphosphate (TSP) and with or without pH adjustment

Treatment	TSP	pH	Description pre-enrichment treatments
1	+	8.5	Regular TSP treatment (no pH adjustment)
2	+	7.0	pH adjusted with HCl to that of control (treatment 4)
3	–	8.5	pH adjusted with NaOH to that of TSP (treatment 1)
4	–	7.0	Control (no pH adjustment)

MATERIALS AND METHODS

Carcass Treatment

In each of 5 trials, 40 prechill broiler carcasses were collected after an inside-outside carcass washer at a commercial processing plant, individually bagged, and transported to the laboratory. Carcasses were divided into 4 treatment groups (Table 1) with 10 carcasses per group. Half of the carcasses were treated with TSP (treatments 1 and 2). The carcasses were dipped 2 at a time in 10% TSP for 5 s at 24°C, allowed to drip for 1 min, and placed into a clean plastic bag, and 500 mL of buffered peptone water[2] (BPW) was added. In treatment 2, the BPW also contained 7.5 mL of 2 *N* HCl to adjust the pH of the pre-enrichment medium to 7.0 during incubation. The other half of the birds were not treated with TSP prior to placement into bags and addition of 500 mL of BPW. In treatment 3 the BPW contained 4.5 mL of 2 *N* NaOH to adjust the pH of the pre-enrichment media to 8.5 during incubation. Treatment 4 was the control in which the BPW was unadjusted at a pH of 7.0. All carcasses were shaken for 1 min, and 5 mL of rinsate was removed from each for the pre-enrichment pH determination followed by incubation of the carcasses in the pre-enrichment rinsate at 37°C for 24 h.

In preliminary tests, the pH of the TSP-treated carcasses in 500 mL of buffered peptone was found to be consistently around 8.5. Untreated carcasses incubated in 500 mL of BPW consistently had a pH of approximately 7.0. In these preliminary trials, it was found that it took 7.5 mL of 2 *N* HCl to adjust the pH of the BPW with the TSP-rinsed carcasses to approximately 7.0 prior to pre-enrichment incubation. It was also found that 4.5 mL of 2 *N* NaOH were required to elevate the pH of BPW to approximately 8.5 prior to pre-enrichment incubation of a non-TSP treated or control carcasses. This design allowed for direct comparison of TSP treatment with non-TSP–treated carcasses at neutral pH and pH values of 8.5.

Salmonellae Sampling

Carcasses were sampled using a modified whole carcass enrichment method (Cox and Blankenship, 1975; Simmons et al., 2003a,b; Bourassa et al., 2004; Bystroń et al., 2004). After incubation for 24 h at 37°C, each bag containing a carcass and 500 mL of BPW was shaken for 10 s. One-tenth milliliter of rinsate was transferred to 10 mL of Rappaport-Vassiliadis broth,[2] and 0.5 mL rinsate was transferred to 10 mL TT (Hajna) broth[2] and incubated 24 h at 42°C. Each broth was then streaked onto brilliant green-sulfa[2] and modified lysine iron[3] agar and incubated 24 h at 35°C. Triple sugar iron[2] and lysine iron[2] agar slants were inoculated with suspect salmonellae colonies and incubated for 24 h at 35°C. Poly O[2] and Poly H[4] agglutination tests were used to confirm presumptive positives as salmonellae.

Statistical Analysis

Salmonellae recovery data were analyzed using the chi-squared test procedure. Pre-enrichment pH data were analyzed using the GLM procedure of SAS software (SAS Institute, 1998). Sources of variation were treatment (4) and trial (5). For all analyses significance was determined at $P < 0.05$, and the mean square error was the error test statistic.

RESULTS AND DISCUSSION

Pre-Enrichment pH

The results for the pre-enrichment rinsate pH of the 4 treatments prior to incubation are presented in Table 2. The mean pH across the 5 trials for the TSP, unadjusted pH treatment (treatment 1) was 8.44 and for the non-TSP

TABLE 2. Mean pH value of the preincubation, pre-enrichment rinsate from carcasses rinsed or not rinsed with trisodium phosphate (TSP) and following pH adjustment to the alkaline pH of TSP or the neutral pH of buffered peptone water

	Treatment[1]				
	1	2	3	4	
	TSP		No TSP		Mean square error
Trial	Alkaline	Neutral	Alkaline	Neutral	
1	8.62	7.48	8.64	7.11	
2	8.53	7.30	8.50	7.03	
3	8.35	7.06	8.72	7.18	
4	8.15	7.02	8.81	7.18	
5	8.55	7.33	8.60	7.11	
$\bar{x}$	8.44[b]	7.24[c]	8.65[a]	7.12[c]	0.07

[a–c]Values within a row with no common superscripts differ significantly ($P < 0.05$).

[1]n = 10 per mean.

[2]Becton Dickinson, Sparks, MD.
[3]Oxoid, Basinstoke, Hampshire RG24 8PW, UK.
[4]Microgen, Camberly, Surrey GU15 3DT, UK.

TABLE 3. Recovery of salmonellae from broiler carcasses (number positive / number tested) from carcasses treated or not treated with trisodium phosphate (TSP) and incubated at neutral or alkaline pH

	Treatment			
	1	2	3	4
	TSP		No TSP	
Trial	Alkaline	Neutral	Alkaline	Neutral
1	3/10	1/10	2/10	0/10
2	6/10	5/10	9/10	7/10
3	10/10	10/10	10/10	10/10
4	1/10	4/10	4/10	0/10
5	0/10	2/10	2/10	2/10
Total	20/50	22/50	27/50	19/50
Probability				
Treatment	0.4222			
Pre-enrichment	0.2225			

treated, unadjusted pH control (treatment 4) was 7.12. The neutralized TSP treatment (treatment 2) had a preincubation mean pH of 7.24, which was not significantly different from the control pH of 7.12. The non-TSP, alkaline rinse (treatment 3) had a mean pH of 8.65, which was significantly greater than the pH of 8.44 for the TSP, nonadjusted (treatment 1) rinsate. The TSP, nonadjusted and non-TSP, adjusted (treatments 1 and 3, respectively) were significantly greater than the pH of the TSP, adjusted pH, and control (treatments 2 and 4, respectively) pre-enrichment rinse.

These results show that the pH adjustment of the TSP-treated carcasses and the control carcasses pre-enrichment media was successful. However, the pH of the non-TSP, alkaline-adjusted pre-enrichment medium was significantly greater than the pH of the TSP, nonadjusted medium. *Salmonella* will grow within a pH range of 4.5 (minimum) and 9.5 (maximum) (Bell and Kyriakides, 2002). Therefore, the significant difference found between treatments 1 and 3 of only 0.2 pH units is unlikely to have affected salmonellae growth and recovery.

Salmonellae Recovery

Data for recovered salmonellae by TSP treatment and pH adjustment of the pre-enrichment rinsate are reported by number of salmonellae-positive carcasses tested in each trial and treatment (Table 3). There was wide variation among trials with trial 3 exhibiting 100% of the carcasses as salmonellae positive, where as trials 1 and 5 were only 15% positive. Neither main effects of TSP treatment nor pH adjustment (8.5 vs. 7.0) had any significant effect on salmonellae recovery. Salmonellae were detected from 40% of the TSP alkaline pre-enrichment carcasses, 44% of the TSP neutral pre-enrichment carcasses, 54% of the non-TSP alkaline pre-enrichment carcasses, and 38% of the non-TSP neutral pre-enrichment carcasses (Table 3).

The lack of salmonellae reduction between the TSP-treated and non-TSP treated carcasses might have been due to the minimal time of the pH shock (<2 min from dip to pre-enrichment), a lack of an immersion chilling step, or the high sensitivity of the method of salmonellae recovery. The high pH shock (pH ~ 12) from the initial 5-s TSP treatment and 1 min of resident time might not have a significant effect on salmonellae recovery. Similar short-term pH shock results were found in a study reported by Teo et al. (1996) in which only minimal reductions of *Salmonella enteritidis* cells were detected after 1 min of exposure to pH 11. In another study, a 6-s dip in 10% TSP also did not decrease salmonellae recovery from broiler carcasses (Ellerbroek et al., 1996). Therefore, in the current study, the rapid exposure of the TSP-treated birds to the BPW, followed immediately by shaking, might have limited the direct pH shock effect of TSP on the salmonellae. Therefore, a pH shock effect would not be as pronounced as in commercial processing and chilling environments or in those previously reported research studies in which TSP exposure was longer or not ameliorated by buffers or pH adjustment.

The lack of an immersion chilling step that would have had a washing or scrubbing action on the carcass may have prevented loose or detached salmonellae from being physically removed from the carcass prior to enrichment. In a previous study, TSP treatment significantly decreased salmonellae recovery in comparison with untreated controls when the methods of TSP treatment and salmonellae detection were the same as in the present study, except carcasses were rinsed off and immersion chilled (in an ice and water mixture) between TSP treatment and salmonellae recovery (Bourassa et al., 2004).

Whole carcass enrichment has been previously reported as a more sensitive salmonellae recovery method than the rinse aliquot method (Cox and Blankenship, 1975; Simmons et al., 2003a,b). Although TSP may have decreased carcass salmonellae numbers, the numbers were not low enough (less than 20 cells per carcass) to prevent detection by the whole carcass enrichment method. This increased sensitivity might have allowed salmonellae to be recovered from TSP-treated carcasses and resulted in ineffectiveness relative to other previous studies (Coppen et al., 1998; Yang et al., 1998; Whyte et al., 2001).

There was also no significant difference in salmonellae recovery between the neutral and alkaline pH pre-enrichments. This result might have occurred because the alkaline pH of 8.5 was not high enough to decrease salmonellae numbers below the level that they would be detected by whole carcass enrichment. According to Bell and Kyriakides (2002), the minimum and maximum pH values for salmonellae proliferation are 3.8 and 9.5, respectively. The pH of 8.5 was within that range; therefore the initial pre-enrichment pH was not likely to have significantly affected salmonellae recovery in comparison with pH 7.1.

These results indicate that salmonellae recovery was not affected by TSP treatment or pH adjustment of the pre-enrichment media. This affect might have been due to limited direct exposure to TSP (no pH shock due to rapid rinsing in the preincubation media), the comparative lack of scrubbing and removal of bacteria that would occur during immersion chilling, or the adjusted final pH of the pre-enrichment media not being sufficiently high to result in a significant decrease in salmonellae recovery.

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REFERENCES

Bell, C., and A. Kyriakides. 2002. *Salmonella*: A Practical Approach to the Organism and its Control in Foods. Blackwell Science, Ames, IA.

Bender, F. G., and E. Brotsky. 1991. Process for treating poultry carcasses to control salmonellae growth. US Patent 5,069,922.

Bender, F. G., and E. Brotsky. 1992. Process for treating poultry carcasses to control salmonellae growth. US Patent 5,143,739. Assignee: Rhone-Poulenc Inc.

Bourassa, D. V., D. L. Fletcher, R. J. Buhr, M. E. Berrang, and J. A. Cason. 2004. Recovery of salmonellae from trisodium phosphate-treated commercially processed broiler carcasses after chilling and after seven day storage. Poult. Sci. 83:2079–2082.

Bystroń, J., K. Kosek-Paszkowska, J. Molenda, and M. Czerw. 2004. Occurrence of *Salmonella* spp. in chicken carcasses. Med. Weter. 60:317–319.

CDC. Center for Disease Control and Prevention, Division of Bacterial and Mycotic Diseases. 2003. Disease information: Salmonellosis. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_t.htm. Accessed March 2004.

Coppen, P., S. Fenner, and G. Salvat. 1998. Antimicrobial efficacy of AvGard carcass wash under industrial processing conditions. Br. Poult. Sci. 39:229–234.

Cox, N. A., and L. C. Blankenship. 1975. Comparison of rinse sampling methods for detection of salmonellae on eviscerated broiler carcasses. J. Food Sci. 40:1333–1334.

Ellerbroek, L., E. M. Okolocha, and E. Weise. 1996. Lactic acid and trisodium phosphate for decontamination of poultry meat. Pages 187–195 in Factors Affecting the Microbial Quality of Meat. M. H. Hinton and C. Rowlings, ed. University of Bristol Press, Bristol, UK.

Giese, J. 1992. Experimental process reduces *Salmonella* on poultry. Food Technol. 46:112.

Giese, J. 1993. *Salmonella* reduction process receives approval. Food Technol. 47:110.

Kim, J. W., M. F. Slavik, and F. G. Bender. 1994a. Removal of *Salmonella typhimurium* attached to chicken skin by rinsing with trisodium phosphate solution: Scanning electron microscopic examination. J. Food Safety 14:77–84.

Kim, J. W., M. F. Slavik, M. D. Pharr, D. P. Raben, C. M. Lobsinger, and S. Tsai. 1994b. Reduction of *Salmonella* on postchill chicken carcasses by trisodium phosphate (NA_3PO_4) treatment. J. Food Safety 14:9–17.

Lee, R. M., P. A. Hartman, D. G. Olsen, and F. D. Williams. 1994. Metal ions reverse the inhibitory effects of selected food-grade phosphates in *Staphylococcus aureus*. J. Food Prot. 57:284–288.

Li, Y., J. W. Kim, M. F. Slavik, C. L. Griffis, J. T. Walker, and H. Wang. 1994. *Salmonella typhimurium* attached to chicken skin reduced using electrical stimulation and inorganic salts. J. Food Sci. 59:23–25.

Lillard, H. S. 1994. Effect of trisodium phosphate on salmonellae attached to chicken skin. J. Food Prot. 57:465–469.

Sampathkumar, B., G. G. Khachatourians, and D. R. Korber. 2003. High pH during trisodium phosphate treatment causes membrane damage and destruction of *Salmonella enterica* serovar enteritidis. Appl. Environ. Microbiol. 69:122–129.

SAS Institute. 1998. SAS User's Guide. Version 8. SAS Institute Inc., Cary, NC.

Simmons, M., D. L. Fletcher, M. E. Berrang, and J. A. Cason. 2003a. Comparison of sampling methods for the detection of *Salmonella* on whole broiler carcasses purchased from retail outlets. J. Food Prot. 66:1768–1770.

Simmons, M., D. L. Fletcher, J. A. Cason, and M. E. Berrang. 2003b. Recovery of *Salmonella* from retail broilers using a whole carcass enrichment procedure. J. Food Prot. 66:446–450.

Somers, E. B., J. L. Schoeni, and C. L. Wong. 1994. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. Int. J. Food Microbiol. 22:269–276.

Teo, Y. L., T. J. Raynor, K. R. Ellajosyula, and S. J. Knabel. 1996. Synergistic effect of high temperature and high pH on the destruction of *Salmonella enteritidis* and *Escherichia coli* O157:H7. J. Food Prot. 59:1023–1030.

Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony. 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. J. Food Prot. 64:179–183.

Yang, Z., Y. Li, and M. Slavik. 1998. Use of antimicrobial spray applied with an inside-outside birdwasher to reduce bacterial contamination on prechilled chicken carcasses. J. Food Prot. 61:829–832.